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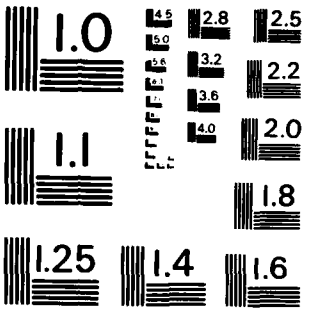

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BRAIN AND LIVER GLUTAMINE SYNTHETASE OF RANA CATESBEIANA AND RANA CANCRIVORA

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JULY 1983
FINAL REPORT

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DEAN OF THE FACULTY
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Editorial Review by Captain Hale
Department of English
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Thomas E McCann

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)
Enzyme assays using the gamma-glutamyl transferase method provided estimates of glutamine synthetase activity in brain and liver tissues of Rana catesbeiana and Rana cancrivora. Glutamine synthetase specific activity in liver and brain tissues were not significantly different between these species. In the salt-water frog R. cancrivora, liver glutamine synthetase activity is insufficient to produce enough glutamine to supply the urea cycle with a nitrogen source. The pathway involving glutamine as a nitrogen source for urea production in marine chondrichthian fishes is not valid in the marine amphibian, Rana cancrivora.

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INTRODUCTION

Previous work has determined that glutamine synthetase plays a role in the synthesis of urea in marine chondrichthian liver (1,15,16). This group of marine urea-retaining animals utilizes the urea as an osmolyte to counter the osmolarity of their environment (2,7,8,10,14). Figure 1 shows an evolutionary tree of vertebrates. The relationship between urea retention as an osmolyte in marine species and presence of high levels of glutamine synthetase in the liver is clear for most groups. The lungfishes (Dipnoids) do not retain urea except to avoid ammonia toxicity during estivation (11). This mechanism is not osmoregulatory and the group does not live in salt-water.

The coelacanth, Latimeria chalumnae, in the Class Osteichthyes, is a marine fish that also retains urea as an osmolyte (3,12). It too has a relatively high level of glutamine synthetase in its liver (16). The proposed connection between glutamine synthesis and urea synthesis by these marine species was discussed by Webb (15) and is depicted in Figure 2. The key enzymes of this pathway, glutamine synthetase and glutamine-dependent carbamoyl phosphate synthetase III, were shown later in a similar figure by Anderson (1).

The saltwater, crab-eating frog, Rana cancrivora, retains urea for the same purpose (9). Although Rana cates-

beiana, fresh-water bullfrogs, switch from ammonioteleism to ureotelism (4,5,6) they do not retain urea in their plasma and tissues. The question remained as to whether the salt-water frogs also use the pathway in Figure 2 as a means of scavenging ammonia for urea synthesis. This question is addressed here by analyses of the liver and brain glutamine synthetase of Rana catesbeiana and Rana cancrivora. The methods and types of comparisons between fresh-water and salt-water species used previously (15,16) were unchanged for this study.

MATERIALS AND METHODS

Rana catesbeiana, bullfrog tadpoles and adult specimens were obtained from several sources (15). Rana cancrivora, salt-water, crab-eating frogs, were obtained from Professor Angel C. Alcala, Silliman University, Philippines.

Tissues were removed from the live specimens and homogenated with water immediately prior to the assays. The Rana cancrivora specimens had been placed in full-strength artificial sea water and were highly stressed. Several of the salt-water frogs had died and the tissues from the three remaining, barely living specimens were removed, sealed in foil, and frozen. These tissues were assayed five days later.

The tissue homogenates were assayed for glutamine syn-

thetase activity according to the method of Webb (15) and Webb and Brown (16) under the following conditions: Ten minutes of incubation at 25°C; pH 6.4; 2 ml incubation mixture containing 60 mM L-glutamine, 15 mM hydroxylamine-HCl, 0.4 mM Na_2ADP; 20 mM KH_2AsO_4, 3 mM $MnCl_2$, and 40 mM imidazole. The gamma-glutamyl hydroxamate produced by the transferase enzyme activity was complexed with $FeCl_3$ (in HCl) and compared to a gamma-glutamyl hydroxamate standard (Sigma Chemical Co.) at 500nm with a B&L Spectronic 20 Spectrophotometer. A unit of glutamine synthetase activity is defined as the production of one micro-mole of gamma-glutamyl hydroxamate per minute at 25°C. Protein content was determined by the biuret method described by Zamenhof (17) using a bovine albumin standard (Calbiochem-Behring #126575).

RESULTS AND DISCUSSION

There was no significant difference between the specific activity of glutamine synthetase in the liver of urea-retaining versus non-urea-retaining species of amphibians sampled in this study (Table 1). The ratio of liver to brain specific activity also showed no difference as had been expected on the basis of an analogous study in fish (16). In that study, the urea-retaining fish had a liver to brain specific activity ratio of 1.55 $\pm$ 0.61(19) as compared

to 0.02 $\pm$ 0.02(48) for non-urea-retaining fish. This very significant difference in fish was not evident in the current study on amphibians. Activity in kidney tissue was also extremely low in both species of amphibians.

Several explanations of these unexpected data are possible. The salt-water frogs may have been over-stressed by immersion in full-strength artificial sea water and the activity of glutamine synthetase in the liver of these moribund frogs may have disappeared as a result. This however, is not consistent with an earlier report on stability of glutamine synthetase in the liver of urea-retaining fish (15). The same argument applies to the possibility of loss of enzyme activity from the time of sacrifice to the time of assay; particularly in view of the retention of activity in the brain tissue which was treated in the same manner as the liver tissue.

Another possibility involves control of the pathway of urea synthesis. Perhaps glutamine synthetase is subject to sensitive control in the liver of salt-water frogs. These specimens were shipped in a box lined with sponges soaked in fresh-water. Their exposure to the fresh-water may have been sufficient to activate a potential regulatory mechanism to stop urea synthesis since it was not needed at that time as an osmoregulatory solute. Glutamine synthetase could be the first enzyme to be repressed in the pathway of urea

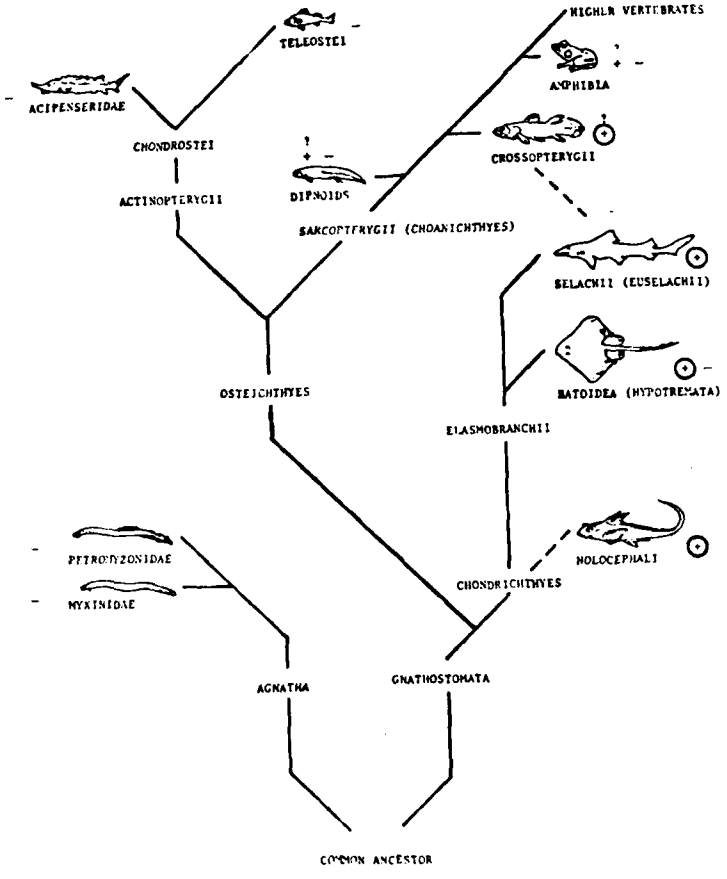
synthesis (see Figure 2). Lack of reactivation of the pathway on immersion in full-strength artificial sea water could have been due to osmotic shock.

One more explanation could be the presence of a functional carbamoyl phosphate synthetase I in the salt-water frog liver which produces carbamoyl phosphate using ammonia as a substrate in place of glutamine. This possibility raises questions about the ability of the carbamoyl phosphate synthetase I to produce enough carbamoyl phosphate to feed the urea cycle at a sufficient rate to produce the high levels of osmoregulatory urea found in the salt-water frogs.

SUMMARY

Glutamine synthetase activity in liver and brain tissues from salt-water frogs (Rana cancrivora) closely resembles the activity in fresh-water frogs (Rana catesbeiana). Problems with maintaining the salt-water frogs may have lowered their liver tissue activity but probably not enough to reverse the relationship seen here. In the salt-water frog R. cancrivora, liver glutamine synthetase activity is insufficient to produce enough glutamine to supply the urea cycle with a nitrogen source. The pathway previously proposed which involved glutamine as a nitrogen source for urea production in marine chondrichthian fishes is apparently not valid in the marine amphibian, Rana cancrivora.

FIGURE 1--Evolutionary tree of vertebrates



The occurrence of high levels of glutamine synthetase in liver is indicated by presence of a circle by the group. The presence (+) or absence (-) of urea retention is shown to indicate correlations. Format after the style of Romer (13).

FIGURE 2--Proposed pathway for assimilation of ammonia into urea in marine Chondrichthyes liver (from Webb (15))

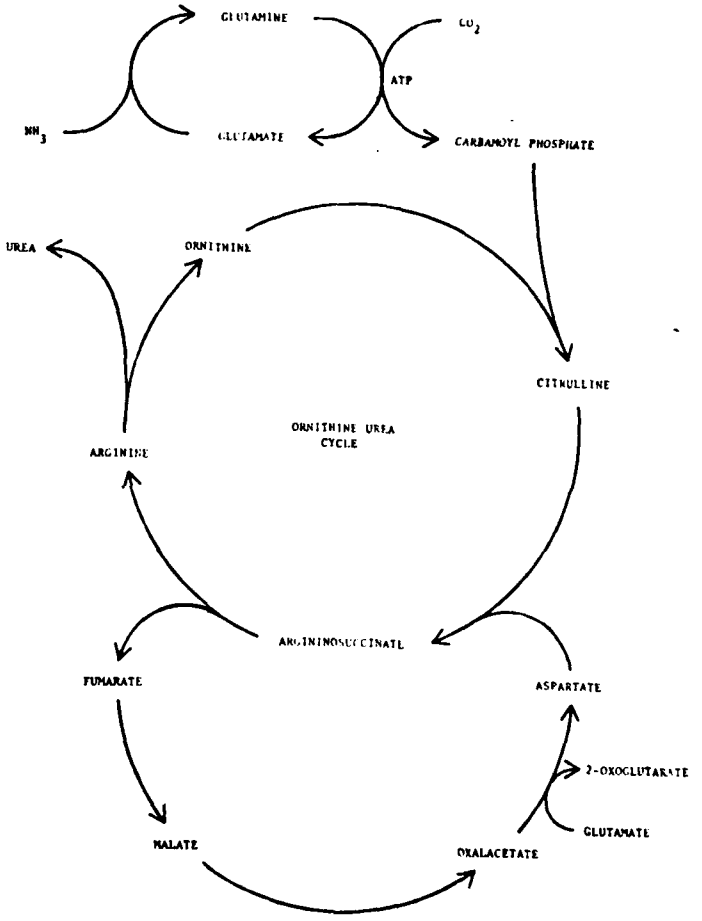


TABLE 1--Liver and brain glutamine synthetase of urea-retaining and non-urea-retaining amphibians.

Species[1]	Specific[2] activity		Specific[2] activity ratio
	Liver	Brain	$\frac{\text{Liver}}{\text{Brain}}$
Non-urea-retaining	0.00 ± 0.00(9)	0.62 ± 0.15(9)	0.01 ± 0.01(9)
Urea-retaining	0.00 ± 0.00(2)	0.45 ± 0.13(3)	0.01 ± 0.01(2)

[1]Non-urea-retaining amphibians: Rana catesbeiana bullfrogs (data from Webb ('79)). Urea-retaining amphibians: Rana cancrivora, salt-water, crab-eating frogs.

[2]Mean of activities is expressed as units of glutamine synthetase activity per mg protein ± standard deviation. Number of specimens is indicated parenthetically.

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